Narrative review

An update on the detection of tuberculosis through molecular testing

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Actualización en la detección de la tuberculosis a través de pruebas moleculares

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Abstract
Tuberculosis is a respiratory infectious disease that affects one third of the world's population and is a significant threat to global health. Detecting tuberculosis early is crucial for effective treatment and preventing its spread. One solution to improve diagnosis and address antituberculosis drug resistance is the use of high-throughput molecular tests for the identification of *Mycobacterium tuberculosis* and its susceptibility. This narrative review study seeks to describe the generalities, efficacy, sensitivity, advantages and limitations of the main molecular tests: Truenat® MTB, MTB plus and MTB-RIF, Abbott RealTime MTB and MTB RIF/INH on the m2000sp and m2000rt system and FluoroType MTBDR, and to compare them with GeneXpert MTB/RIF or Xpert Ultra, used for the detection of the tuberculosis drug-resistant pathogen. These tests use various techniques for the detection of *Mycobacterium tuberculosis* DNA and quantification of bacterial load with high sensitivity and specificity, rapid results, reduction of human error, as well as early detection of drug-resistant strains.

Keywords
Mycobacterium Tuberculosis, Tuberculosis, Diagnosis, Molecular Tests.

Resumen
La tuberculosis es una enfermedad infecciosa respiratoria que afecta a un tercio de la población mundial y es una amenaza significativa para la salud global. La detección de la tuberculosis de manera temprana es crucial para un tratamiento eficaz y prevenir su propagación. Una solución para mejorar el diagnóstico y abordar la resistencia a los medicamentos antituberculosos es el uso de pruebas moleculares de alto rendimiento para la identificación del *Mycobacterium tuberculosis* y su susceptibilidad. Este estudio de revisión narrativa busca describir las generalidades, la eficacia, la sensibilidad, las ventajas y las limitaciones de las principales pruebas moleculares; Truenat® MTB, MTB plus y MTB-RIF, Abbott RealTime MTB y MTB RIF/INH en el sistema m2000sp y m2000rt y FluoroType MTBDR, además, de compararlas con GeneXpert MTB/RIF o Xpert Ultra, utilizadas para la detección del patógeno resistente a medicamentos tuberculosos. Estas pruebas utilizan diversas técnicas para la detección del ADN del *Mycobacterium tuberculosis* y la cuantificación de la carga bacteriana con alta sensibilidad y especificidad, resultados rápidos, reducción de los errores humanos, así como la detección temprana de cepas drogo-resistentes. A pesar de que requieren infraestructura especializada y competencias profesionales para su implementación, representan avances significativos con el potencial de mejorar la atención sanitaria y la gestión de la tuberculosis. Estas pruebas moleculares, comparadas con el GeneXpert, son una alternativa viable, aunque esta última tecnología sigue siendo la preferida en áreas con recursos limitados.

Palabras clave
Mycobacterium tuberculosis, tuberculosis, diagnóstico, técnicas de diagnóstico molecular.

Introduction

Tuberculosis (TB) is an infectious disease caused by the *Mycobacterium tuberculosis* (MTB) complex that encompasses a group of closely genetically related species, including MTB, the most recognized as it is responsible for infecting more than one-third of the world's human population[i]. TB continues to pose a considerable health risk globally, as indicated by figures provided by the World Health Organization (WHO), which estimated 7.5 million cases diagnosed with TB in 2023 and a total of 1.3 million deaths from TB. In addition, it is believed that around three million TB cases went undetected during the same year.[i]

Early diagnosis of TB is critical for effective treatment and prevention of disease spread; likewise, detection of drug resistance is important for effective treatment.[i] A potential solution to close the diagnostic gap for TB and drug resistance lies in using centralized high-throughput platforms to detect MTB through molecular drug susceptibility testing.[i,ii] Several molecular technologies are available, such as nucleic acid amplification tests, whole genome sequencing, and the GeneXpert system (Xpert MTB/RIF or Xpert MTB/RIF Ultra). These detect the presence of MTB and its antibiotic resistance genes by DNA detection in patient samples.[i-iii]

The GeneXpert system is a standard and widely used molecular test that rapidly detects MTB and assesses resistance to multiple anti-TB drugs, primarily rifampicin (RIF), the first-line drug for TB. Other molecular tests such as Truenat MTB, Abbott RealTime MTB, and FluoroType MTB are also used in the diagnosis of TB.[i-iv]

These molecular tests provide greater speed and accuracy compared to traditional methods such as microscopy and culture. In addition, they allow the detection of antibiotic resistance, which facilitates more accurate and effective treatment.[v]

Through this review, the generalities, efficacy, sensitivity, advantages, and limitations of the Truenat MTB, MTB plus and MTB-RIF, Abbott RealTime MTB and MTB RIF/INH tests on m2000sp and m2000rt systems, and FluoroType MTBDR, as well as their comparison with GeneXpert MTB/RIF or Xpert® MTB/RIF Ultra, used for the detection of MTB resistant to tuberculous drugs, are described.

Discussion

Test Overview

Truenat MTB, MTB Plus and & MTB-RIF Dx

The Truenat MTB, MTB Plus and MTB-RIF Dx tests are molecular tests for the detection of MTB and RIF resistance,[vi] developed by Bigtec Labs, Molbio Diagnostics of Goa, India and endorsed by WHO in 2020.[iv]

Truenat testing employs loop-mediated isothermal amplification technology to identify the presence of MTB and assess resistance to RIF. It consists of a technique of nucleic acid amplification at a constant temperature, usually around 65 °C. In addition, it is semi-quantitative; it can indicate the approximate amount of bacteria present directly in sputum samples. The devices for these tests are portable. The tests are capable of performing simultaneously multiple reactions, which facilitates faster and more efficient diagnosis.[vii]

The Truenat MTB test is the basic test used for the identification of the presence of MTB. The Truenat Plus assays, an enhanced version of the Truenat MTB, detect specific genes such as nrdB, nrdZ, and IS6110.[viii] The nrdB gene encodes the beta subunit of ribonucleotide reductase, a crucial enzyme that supplies the precursors necessary for DNA synthesis. It also detects the nrdZ gene, which is part of the dormancy-associated gene regulon. In addition, it detects the IS6110 gene, which is used as a specific epidemiological marker for TB and is found exclusively in the MTB complex.[ix,x]

Likewise, Truenat MTB-Rif Dx tests are able to detect mutations in the *rpoβ* gene, which encodes the beta subunit of RNA polymerase in bacteria and is the main target of action of RIF. Mutations in the *rpoβ* gene can alter the structure of RNA polymerase, preventing RIF from efficiently binding to the active site of the enzyme and is ineffective in inhibiting DNA-to-RNA transcription in bacteria with *rpoβ* mutations, leading to resistance to RIF. Test results are obtained in less than one hour.[xi-xiii]

Abbott RealTime MTB and MTB RIF/INH

Abbott RealTime MTB is a molecular test launched by Abbott Company in 2015 to detect MTB, endorsed by WHO since its development. This test also identifies RIF and isoniazid (INH)-resistant variants, expanding its utility in diagnosing antibiotic-resistant strains.[xiv] It employs the real-time polymerase chain reaction (PCR-RT) technique, which focuses on identifying the gene encoding the antigen B protein, essential in the synthesis of mycolic acids. These are key components of the bacterial cell wall and are crucial for survival and resistance to host immune responses.[xv] The IS6110 gene is important in the identification of MTB, as a transposon-like element, is mobile within the bacterial genome and with specific characteristics of the bacterial complex; however, the number of copies of the IS6110 element may vary between different strains of the bacterium.[xvi]

The Abbott Realtime MTB RIF/INH test can detect resistance to RIF (*rpoβ* gene) and INH (*katG* and *inhA* genes). Moreover, the *katG* gene encodes catalase-peroxidase enzymes that are necessary to activate INH within the bacteria. If there is a mutation in the *katG* gene, INH will not be activated and will not be able to perform its antimicrobial actiona.[xvii] On the other hand, the *inhA* gene encodes the enzyme enoyl-ACP reductase, essential for the synthesis of fatty acids in bacteria, including the synthesis of

bacterial cell walls. When there are mutations in the *inhA* gene, the enzyme enoyl-ACP reductase can become less sensitive to inhibition by INH, and consequently generates resistance.[xviii]

The Abbott RealTime MTB and MTB RIF/INH tests differ in technology, sample types and processing time in the m2000sp and m2000rt systems. The m2000sp system uses PCR-RT, while the m2000rt uses PCR-RT with fluorescent probes that are validated for sputum samples. The m2000rt also supports bronchoalveolar lavage and pleural fluid samples. The processing time for m2000sp is approximately two hours, while m2000rt is four hours.[xiv,xvi,xix]

FluoroType MTBDR

Fluorotype MTBDR1.0 and MTBDR2.0 are WHO-approved molecular tests for the detection of MTB, and were developed by the German laboratory Hain Lifescience in 2019. These tests use linear after exponential PCR (LATE-PCR) technology, and special probes with lights-on/lights-off detection. In addition, they incorporate LiquidArray technology, a PCR platform that enables the detection of multiple targets in a single reaction through the use of specific primers. These tests are designed to identify mutations in the *rpoβ*, *inhA* and *katG* genes, which indicate drug resistance in multidrug-resistant TB cases.[v]

FluoroType MTB VER 1.0 and FluoroType MTB VER 2.0 have key similarities and differences. Version 1.0 focuses on the IS6110 insertion element and can process decontaminated pulmonary and extrapulmonary samples. In contrast, version 2.0 targets the *rpoβ* gene to detect MTB complex and RIF resistance, the *inhA* promoter and the *katG* gene for INH resistance, validated for respiratory samples. Both versions employ LiquidArray technology in PCR amplification, providing results in an average of two hours and 30 minutes. Notably, version 1.0 uses FluoroCycler® 12, while version 2.0 uses FluoroCycler® XT, thermal cyclers designed specifically for this test. The entire process can be completed in an average of two and a half hours.[xx,xxi]

Sensitivity and specificity

Truenat MTB, MTB Plus and MTB-RIF Dx

In a four-hospital study in Cameroon involving 945 people with TB symptoms, the Truenat MTB Plus test demonstrated a sensitivity of 91 % (228 of 251) in TB patients with bacteriological confirmation of disease by culture. The overall specificity of the Truenat MTB Plus test was 96 %; 31 of the 694 participants with culture-negative TB results were positive for MTB with the Truenat MTB Plus test. Therefore, they concluded that these results support the effectiveness of the Truenat tests as they demonstrate their ability to adequately identify this disease in the majority of cases.[xxii]

Abbott RealTime MTB and MTB RIF/INH

Abbot Realtime MTB has a high specificity (97 %) and sensitivity (93 %), according to information provided by the company.[xxiii] In terms of sensitivity, a study conducted in Africa showed that the Abbot Realtime MTB test has a sensitivity of 92.4 %.[xvi] This study performed such a test on people who already have a confirmed TB diagnosis: in them, the test detected MTB in 73 out of 79 people. In addition, it showed that the test has a specificity of 95.4 %.[xvi] These findings indicate that the Abbott RealTime MTB and MTB RIF/INH tests are highly effective diagnostic resources for TB, with the ability to identify accurately in most clinical situations.[xvi]

FluoroType MTBDR

The FluoroType MTB and MTBDR VER 2.0 tests were evaluated for accuracy in detecting drug-resistant TB in a study conducted at the National Reference Laboratory for Mycobacteria in Borstel, Germany. Of the 610 patients, 360 samples tested positive for MTB on Xpert Ultra, and 250 samples tested negative for MTB on Xpert Ultra. Therefore, FluoroType MTB VER 2.0 showed a sensitivity for manual DNA extraction of 91.6 % and a sensitivity of 89.8 % for automated extraction. Automated DNA extraction had a sensitivity of 92.1 % in contrast to non-automated extraction with 87.7 %. Consequently, the sensitivity for INH was 91.7 %, and for RIF, 98.9 %.[xxiv].

Studies were conducted in South Africa to evaluate the specificity of the FluoroType MTBDR test for the detection of MTB and resistance to RIF and INH. Sputum samples from patients who had a previous evaluation with Xpert MTB/RIF were used, and the results showed a specificity of 100 % for both MTB detection and RIF and INH resistance.[xxv,xxvi]

Advantages

Truenat MTB, MTB Plus and MTB-RIF Dx

Truenat MTB, MTB Plus, and MTB-RIF Dx tests offer several significant advantages over other TB diagnostic methods. They provide

rapid results in less than one hour, allowing immediate initiation of TB treatment. Additionally, they are highly sensitive and specific, enabling the differentiation of TB from other diseases with similar symptoms. Their portability and ease of use make them suitable for primary care settings. These combined advantages make Truenat tests an effective, rapid, and accurate tool for TB diagnosis.[xxv]

Abbott RealTime MTB and MTB RIF/INH

One of the advantages offered by Abbott Realtime MTB and MTB RIF/INH is the ability to process a variability of samples including sputum, bronchoalveolar lavage and already extracted MTB DNA samples.[xxvi]

The m2000sp/m2000rt systems offer complete automation for MTB amplification and detection. These systems allow adjustment of sample volume and number of samples, reduce reagent waste and optimize throughput. In addition, they can process up to 96 samples with reduced turnaround time, most useful in antibiotic resistance testing such as RIF/INH. The capacity is 96 samples in eight hours and the inactivation reagent reduces the risk of contagion during sample handling. The test is noted for its accuracy, with the presence or absence of infection in processed samples.[xxvi-xxviii]

FluoroType MTBDR

FluoroType MTBDR has several significant advantages. It processes sputum samples, which are minimally invasive and easy to obtain. The automated system can analyze up to 94 samples in three hours. Results are automatically generated. This system is characterized by detecting INH resistance and provides crucial information for treatment strategy. Automation reduces the risk of contamination, with a preparation time of 30 minutes. In summary, FluoroType MTBDR offers a fast and accurate assay for drug resistance detection in MTB.[xxvi,xxvii]

Limitations

Truenat MTB, MTB Plus and MTB-RIF Dx

The Truenat MTB, MTB Plus, and MTB-RIF Dx tests, while valuable tools in the diagnosis of TB, have certain limitations to consider, including the possibility of providing false negative results in cases of RIF-resistant TB, because the Truenat MTB-RIF Dx test focuses exclusively on identifying genetic changes in the *rpoβ* gene. Similarly, in cases of low bacterial load, the tests can yield false negatives because they require a minimal amount of MTB DNA to obtain a positive result (five copies of MTB genome and 131 CFU/mL in expectoration samples).[xxii].

Abbott RealTime MTB and MTB RIF/INH

Less common MTB strains may give false negative results due to the difficulty of detection. Also, amplification reagents have a limited shelf life of 90 days from the date of manufacture or 60 days from the date of shipment, which may result in the loss of unused reagents. In addition, maintenance of the units must be performed through contracts with Abbott, which limits the ability to reduce operating costs by performing maintenance through third parties. Finally, the m200sp and m200rt systems do not have a USB port, so data must be manually exported or digitized.[xxviii]

Also, training on using the m200sp and m200rt systems is more complex compared to other TB diagnostic platforms. Training lasts at least five days and includes both theoretical and practical aspects. In addition, the implementation of these platforms requires a robust infrastructure, including isolated spaces for sample preparation.[xxviii]

FluoroType MTBDR

The FluoroType MTBDR has several limitations. Its accuracy in diagnosing TB in low bacterial load samples is limited, as its detection range of 10 398 copies is three times greater than Xpert MTB/RIF. In addition, it does not detect certain mutations related to INH resistance, such as *katG* S315N. Although it has a high detection capability for RIF and INH resistance mutations, there are other tests with even higher sensitivity percentages. Finally, its sensitivity in the diagnosis of extrapulmonary tuberculosis is significantly lower than for sputum samples; because of this, it is not recommended for this type of tuberculosis.[xxi,xxvii,xxviii]

Comparison with GeneXpert

Truenat MTB, MTB Plus and MTB-RIF Dx

The Truenat MTB and MTB PLUS test has lower sensitivity and higher specificity than the Xpert MTB/RIF for TB, especially in smear-negative and HIV-positive subjects. Truenat has a higher specificity than Xpert MTB/RIF in subjects with a history of TB. In terms of identifying RIF resistance, Xpert MTB/RIF has superior sensitivity and specificity compared to Xpert MTB/RIF. Positive results at the trace level are common with the Truenat MTB and MTB PLUS. The Truenat MTB, MTB Plus and MTB-RIF Dx

tests are effective molecular diagnostic tests for tuberculosis and are noted for their rapidity, sensitivity and specificity. These tests are accessible and inexpensive, suitable for primary care settings. Although the GeneXpert MTB/RIF test is more sensitive in detecting mutations in the *rpoβ* gene, it is a more expensive option and requires a specific PCR-RT system.[xxix]

Abbott RealTime MTB and MTB RIF/INH

Although GeneXpert provides faster results than Abbott's RealTime tests, these are more useful for large workflows because they allow processing of a large number of samples. However, this represents a paradox, as the RealTime test would be beneficial in regions where TB is endemic for fast and accurate results, but requires adjustments in laboratory infrastructure, trained personnel, and is more difficult to implement; these features limit its use.[xxviii,xxix]

Abbott MTB detects MTB, MTB RIF/INH identifies MTB and resistance to RIF and INH, while GeneXpert detects MTB with some models able to identify resistance to RIF and uses a second cartridge that detects resistance to INH, quinolones, kanamycin, capreomycin, among others. In addition, GeneXpert is easier to use in resource-limited settings and requires less technical training. Both tests are highly sensitive for MTB detection, however, GeneXpert has a sensitivity of about 98 % while Abbott has a sensitivity of 92-97 %. For RIF and INH resistance, GeneXpert exhibits a high specificity (between 94 and 98 %), while Abbott has a lower specificity. In addition, the detection limit of GeneXpert is 18 CFU/mL, while Abbott RealTime requires only 17 CFU/mL, giving Abbott RealTime a slight advantage over GeneXpert.[xxx]

FluoroType MTBDR

Although Xpert MTB/RIF remains the standard for molecular diagnostic testing for pulmonary tuberculosis, FluoroType MTBDR tests perform similarly. In terms of sensitivity, both tests are quite similar to Xpert, with a sensitivity of 98 % to MTB and FluoroType MTBDR of 97.9 %. In terms of resistance to RIF, Xpert has a percentage of 95 % and FluoroType 96.9 %. On the other hand, the main difference between the two tests is the detection limits. Xpert MTB/RIF can detect MTB in samples with a minimum load of 3781 copies, whereas FluoroType MTBDR has a minimum load limit of 10 398 copies. Therefore, Xpert MTB/RIF is more useful for detection of tuberculosis in tests with low bacterial load.[xxv,xxvii,xxx]

Conclusion

The Truenat MTB, Abbott RealTime MTB, and FluoroType MTBDR diagnostic tests provide effective and reliable methods for the detection of *Mycobacterium Tuberculosis* and anti-tuberculosis drug resistance. Each of these tests has unique advantages, allowing the most appropriate test to be chosen based on the specific needs of the testing context. Truenat MTB uses chip-based nucleic acid amplification that can detect MTB in clinical sputum samples with a sensitivity of 91 % and specificity of 96 %. Abbott RealTime MTB employs *in vitro* polymerase chain reaction (PCR) for qualitative DNA detection, with a sensitivity of 92.4 % and specificity of 95.4 %, enabling high-throughput testing. FluoroType MTBDR is a new molecular test that diagnoses TB and RIF drug resistance. It has a sensitivity of 91.7 % for INH, 98.9 % for RIF, and a specificity of 100 %. Currently, GenXpert is the preferred choice for molecular testing for tuberculosis, and its preference is justified. However, with the continued emergence of new technologies, such as these molecular tests, significant advances in TB detection and management are represented, and their implementation could improve healthcare for confirmed or suspected TB cases. Truenat MTB, Abbott RealTime MTB, and FluoroType MTBDR have provided a more comprehensive perspective and have shown promise for the global fight against TB because of their ability to accurately detect MTB and resistance to certain anti-TB drugs such as RIF and INH.

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